Interaction of Huntingtin associated protein 1 (HAP1) with AHI1, a proetin involved in Joubert syndrome

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ABSTRACT

A number of proteins are known to interact with huntingtin (htt), the Huntington's disease (HD) protein. Htt-associated protein 1 (HAP1) was the first identified htt-binding partner. Unlike htt that is ubiquitously expressed, HAP1 is enriched in the brain, and lack of HAP1 in mice leads to early postnatal death. Recent studies suggest that HAP1 participates in intracellular trafficking. In a co-immunoprecipitation experiment, we found that mouse HAP1 forms a stable complex with Abelson helper integration site 1 protein (AHI1), a protein whose nonsense and missense mutations cause Joubert syndrome, which is an autosomal recessive disorder characterized by abnormal brain development. The interaction of HAP1 and AHI1 is verified by GST pulldown and in vitro binding assays. Both HAP1 and AHI1 colocalize to cytoplasmic puncta in cultured cells and in the mouse brain. Depleting the expression of HAP1 also reduces the level of Ahi1 in mouse brains and cultured cells. Moreover, truncated AHI1, which corresponds to one of the mutations in Joubert syndrome, reduces HAP1 levels and inhibits neurite outgrowth in cultured cells. Together, our findings suggest that HAP1 and AHI1 form a stable protein complex, and their interaction may be involved in early brain development and Joubert syndrome.

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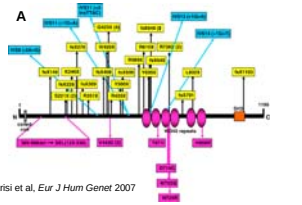
INTRODUCTION

Clinical features of Joubert syndrome (JBTS; JS) include neonatal hypotonia (loss of muscle tone), ataxia, developmental delay, mental retardation, and frquently abnormalities in breathing and eye movements (1). JS is genetically heterogeneous. Among the three subtypes, JBTS3 was found to associate with mutations in the Abelson helper integration site 1 (*AHI1*) gene (2). The protein encoded by this gene (AHI1 or Jouberin) contains seven WD40 repeats, an SH3 domain, potential SH3 binding sites, and an N-terminal coiled–coiled domain. Most mutations of the *AHI1* gene in JS are nonsense or frameshift mutations, which result in truncated N-terminal AHI1 or loss of WD40 and SH3 domains (Fig. 1A; ref. 1). These loss-of-function mutations in AHI1 are consistent with the autosomal recessive nature of JS. However, the role of AHI1 in the pathogenesis of JS remains to be elucidated.



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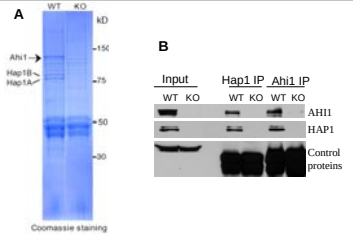
Using immunoprecipitation and mass spectrometry, we found that mouse AHI1 binds tightly to huntingtin-associated protein 1 (HAP1) and forms a stable protein complex in the brain (3). HAP1 was the first identified interacting partner of huntingtin, the Huntington's disease (HD) protein (4). Unlike huntingtin, which is expressed ubiquitously, HAP1 is expressed at variable levels predominantly in brain regions. Mice lacking Hap1 often die at postnatal day 3, suggesting that Hap1 is critical for neonatal development (5). The protein consists of two isoforms spliced alternately that differ only at the C-terminus (i.e., amino acids 579–599 in HAP1A vs. 579–629 in HAP1B). It contains several coiled–coiled domains in the middle rgion, and these may mediate or regulate the interactions of HAP1 with a number of proteins (Fig. 1B; ref. 5).

METHODS & RESULTS



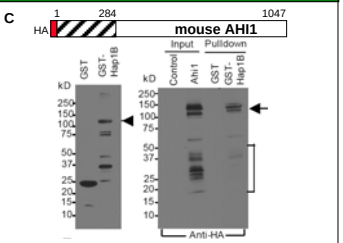


Fig. 2 Association of AHI1 and HAP1

(A) Coomassie staining of HAP1 immunoprecipitates from the brain tissues of wild-type (WT) and HAP1 knockout (KO) mouse pups. Arrow indicates the band that is present with HAP1A and HAP1B in wild-type mouse brain tissue. Mass spectrometry peptide analysis identified this band as AHI1.
(B) Western blots of HAP1 immunoprecipitation confirm the coprecipitation of AHI1 (arrow) with both HAP1A and HAP1B from wild-type mouse brain tissues.
(C) GST and GST-HAP1B were generated, and the intact form of GST-HAP1B is indicated by an arrowhead (left panel). Lysates of HEK293 cells transfected with *AHI1* were pulled down by GST-HAP1 fusion protein. Note that full-length (arrow), but not truncated, AHI1 bound to HAP1.
(D) GFP-HAP1B was pulled down by GST-fused AHI1 truncations (Full-length, MT: WD40 domain and CT: SH3 domain), but barely by NT portion of AHI1.

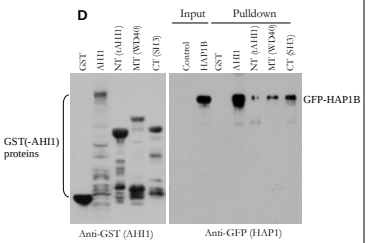


Fig. 3 Coexpression of HAP1 and AHI1 in the same neurons in various brain regions

(A) Immunohistochemical staining of mouse brains with antibodies to HAP1 (upper image) and AHI1 (lower image). Olf: olfactory bulb, Ctx: cerebral cortex, Stra: striatum, Hipp: hippocampus, Cereb, cerebellum, B.S.: brainstem.
(B) Western blots showing the similar distribution of HAP1 and AHI1 in various brain regions. Amy: Amygdala.
(C) Western blot analysis of the expression of HAP1 and AHI1 in embryonic mouse brains at E8, E12, and E18.
(D) Western blots of the cerebellar tissue from mice at different postnatal days (1-30). The blots were probed with antibodies to AHI1, HAP1, and tubulin.

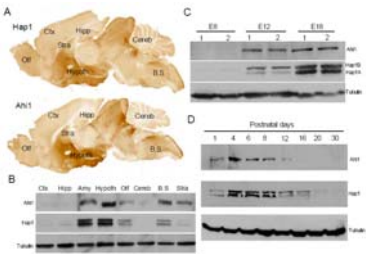
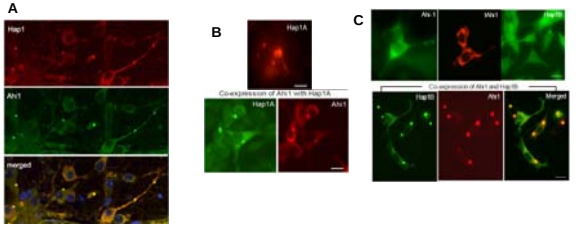


Fig. 4 Ahi1 and Hap1 colocalized in cytoplasmic puncta

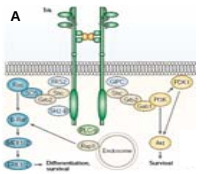
(A) Double immunofluorescent staining of mouse brainstem showing the colocalization of HAP1 and AHI1 in the cytoplasmic puncta.
(B) Co-transfection of HAP1A and AHI1 in HEK293 cells results in the colocalization of both proteins in cytoplasmic puncta.
(C) Expression of AHI1 or HAP1B alone in PC12 cells results in a diffuse distribution of transfected proteins. Co-expression of HAP1B and AHI1 leads to the formation of small cytoplasmic puncta.

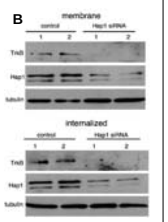


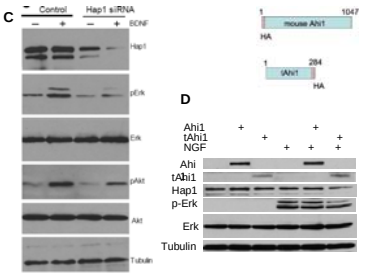
METHODS & RESULTS

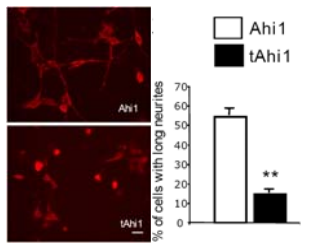
Fig. 5 Truncated AHI1 distabilizes HAP1 and inhibits neurogenesis by disturbing Trk signaling

(A) Trk receptors mediate differentiation and survival signaling through extracellular signal-regulated kinase (ERK) (6).
(B) Western blotting of biotinylated membrane and internalized fractions in cultured brainstem cells showing the decreased level of TrkB when HAP1 expression is suppressed by HAP1 siRNA. Control is adenoviral GFP-infected cells.
(C) Decreased phosphorylation of Erk and Akt in cultured brainstem cells infected by adenoviral HAP1 siRNA.
(D) Truncated AHI1 inhibits NGF-stimulated Erk phosphorylation in PC12 cells.
(E) Transfection of full-length or truncated *AHI1* alone into PC12 cells that were treated with NGF (100 ng/ml for 24 h). Truncated AHI1 inhibits neurite outgrowth of PC12 cells.









DISCUSSION

Stable interaction of AHI1 with HAP1 in vivo

Several lines of evidence indicate that AHI1 and HAP1 form a stable protein complex *in vivo*. First, immunoprecipitation of HAP1 from mouse brain tissue revealed that similar amounts of AHI1 and HAP1 were coprecipitated, suggesting that they are associated with roughly equal stoichiometry. Second, both HAP1 and AHI1 colocalize in cytoplasmic puncta in the brain and in transfected cells. Third, when HAP1 is absent, as in HAP1 knockout mouse brain, the level of AHI1 significantly decreases.

The function of AHI1 and the pathogenesis of JBTS

HAP1 is known to be transported in axons and is important for neurite outgrowth (7). There is mounting evidence that HAP1 is also involved in the internalization of membrane receptors (5, 7). For example, HAP1 stabilizes the level of internalized receptors, such as EGF, GABAA, and TrkA receptors. HAP1 may interact with microtubule-dependent transporters to participate in the internalization, trafficking, and recycling of various membrane receptors (5, 8, 9).

AHI1 contains WD40 repeats and an SH3 domain, which are found in many proteins that participate in cell signaling and intracellular trafficking. The abnormal decussation seen in JBTS reflects defective axonal crossing due to abnormal axonal guidance or neuronal differentiation. The finding that AHI1 binds to HAP1, which is involved in intracellular trafficking and Trk receptor internalization, fits with the idea that AHI1 deficiency in neurons can affect neuronal interaction and networks.

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